Virology **276,** 229–237 (2000)
doi:10.1006/viro.2000.0612, available online at http://www.idealibrary.com on IDEAL®

MINIREVIEW

Beyond Receptor Expression: The Influence of Receptor Conformation, Density, and Affinity in HIV-1 Infection

Robert W. Doms[1]

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received August 16, 2000; accepted August 18, 2000

Enveloped animal viruses enter cells via a series of steps that ultimately result in a fusion reaction between the viral membrane and that of the host cell (Hernandez *et al.,* 1996). Discoveries over the past 4 years have revealed the identities and in some cases the structures of the proteins involved in entry of human immunodeficiency virus type 1 (HIV-1) at the plasma membrane. Each step of the entry process provides information on viral tropism and pathogenesis, and each step is a real or potential target for antiretroviral agents. The rapid expansion of the AIDS pandemic, the high cost and side effects associated with highly active antiretroviral therapy, and the emergence of drug-resistant virus strains call for the development of new interventional strategies. Virus entry is a particularly attractive target since it involves the exposure, at least transiently, of highly conserved domains in Env and depends on cell surface receptors that can be targets for orally available small molecule inhibitors. Therefore, greater understanding of the entry process can have very practical benefits in addition to elucidating factors that impact viral tropism and pathogenesis.

While the molecules involved in HIV-1 entry have been identified, it is clear that there is much more to viral entry than the mere presence of the appropriate receptors on the surface of a target cell. For example, macrophages are an important target cell type *in vivo,* and they express sufficient levels of the viral CD4 receptor as well as the two major HIV-1 coreceptors, CCR5 and CXCR4 (Lee *et al.,* 1999b). However, not all virus strains that require CXCR4 to enter cells can infect macrophages (Rana *et al.,* 1997; Schmidtmayerova *et al.,* 1998; Simmons *et al.,* 1998; Yi *et al.,* 1998). It is not clear why some viruses can utilize CXCR4 expressed on macrophages whereas others cannot. There are other examples of restricted viral entry in either cell lines or primary cell types in which viruses fail to enter cells even though the receptors needed for the membrane fusion reaction are present (Bazan *et al.,* 1998; Dittmar *et al.,* 1997; McKnight *et al.,* 1997; Moriuchi *et al.,* 1997; Schmidtmayerova *et al.,* 1998; Verani *et al.,* 1998; Yi *et al.,* 1998). In addition, there are examples in which increased viral pathogenicity has been associated with relatively subtle changes in the viral envelope (Env) protein, though the mechanisms that account for this are not readily apparent (Cayabyab *et al.,* 1999; Karlsson *et al.,* 1998; Liu *et al.,* 1999; Reimann *et al.,* 1996). Other Env proteins have been described that appear to cause membrane fusion more efficiently than other closely related Env proteins (Etemad-Moghadam *et al.,* 2000; Shieh *et al.,* 2000). The mechanisms for this are also not clear. In this review, I will discuss recent studies that indicate ways in which viruses may differ from one another in the entry process while essentially using the same molecules for the membrane fusion reaction. Specifically, there is now evidence that entry mediated by the HIV-1 Env protein is a highly cooperative process and that it is affected by receptor density as well as by Env-receptor affinities. In addition, some viral receptors exist in antigenically distinct conformations, not all of which may support virus infection equally well. Thus, it is necessary to go beyond receptor expression to fully understand the early steps of HIV-1 infection.

THE PROTEINS INVOLVED IN HIV-1 ENTRY

HIV-1 contains a single type 1 integral membrane protein termed Env that is responsible for both receptor binding and membrane fusion. Initially synthesized as a single polypeptide precursor termed gp160, the protein undergoes a posttranslational proteolytic cleavage that generates a gp120 surface subunit that is noncovalently attached to the gp41 transmembrane domain protein

[1] Address correspondence and reprint requests to the author at Department of Pathology and Laboratory Medicine, University of Pennsylvania, 806 Abramson, 34th and Civic Center Boulevard, Philadelphia, PA 19104. Fax: 215-573-2883. E-mail: doms@mail.med.upenn.edu.

0042-6822/00 $35.00

(Wyatt and Sodroski, 1998). Thus, the topology and processing of HIV-1 Env is similar to many other viral membrane fusion proteins. While attachment of HIV-1 to the cell surface can result from interactions with many surface molecules (Geijtenbeek *et al.,* 2000; Mondor *et al.,* 1998), binding of the gp120 subunit to CD4 is the first required step of virus infection. However, CD4 binding alone is not sufficient for virus infection as it does not trigger the conformational changes needed for membrane fusion. For this to occur, Env must also interact with a coreceptor. All HIV-1 strains studied to date use the chemokine receptors CCR5 (R5 strains), CXCR4 (X4 strains), or both molecules (R5X4 strains) as coreceptors (Alkhatib *et al.,* 1996; Berger *et al.,* 1998; Choe *et al.,* 1996; Deng *et al.,* 1996; Doms *et al.,* 1999; Doranz *et al.,* 1996; Dragic *et al.,* 1996; Feng *et al.,* 1996). R5 virus strains typically infect macrophages and primary T-cells, and are the virus type most commonly transmitted between individuals. X4 virus strains tend to evolve years after infection in a subset of individuals as a consequence of mutations in Env, and infect primary T-cells and transformed T-cell lines (Connor *et al.,* 1997; Scarlatti *et al.,* 1997). Thus, the differential use of the major coreceptors by virus strains coupled with their patterns of expression largely explains viral tropism at the level of entry. The importance of CCR5 for virus transmission was shown by the discovery that individuals who lack CCR5 due to a naturally occurring polymorphism are highly resistant to virus infection, making this receptor an important drug target (Dean *et al.,* 1996; Liu *et al.,* 1996; Samson *et al.,* 1996).

In addition to CCR5 and CXCR4, approximately a dozen other coreceptors have been identified through the use of *in vitro* assays (Choe *et al.,* 1996, 1998; Deng *et al.,* 1997; Doranz *et al.,* 1996; Edinger *et al.,* 1998; Farzan *et al.,* 1997; Liao *et al.,* 1997; Reeves *et al.,* 1997; Rucker *et al.,* 1997; Samson *et al.,* 1998). In general, these alternative coreceptors are used by only a subset of HIV or SIV strains, and they tend to support virus infection less efficiently than the major coreceptors. The *in vivo* relevance of the alternative coreceptors is not clear, with the most telling experiments being those in which virus strains are examined for the ability to infect CCR5-negative human PBMC in the presence of CXCR4 antagonists (Zhang *et al.,* 1998). With one exception (Sharron *et al.,* 2000), infection of CCR5-negative human PBMCs by HIV-1, when it occurs, is dependent upon CXCR4. These studies argue that none of the alternative coreceptors are relevant for infection of the most important target cell types *in vivo.* The reasons for this are likely to be related to expression patterns and levels. The APJ coreceptor, for example, is not expressed at detectable levels on CD4-positive cells (Puffer *et al.,* 2000). Other receptors may function only at very high levels of expression that are not attained *in vivo.* There is some evidence that the CCR8 coreceptor may support virus infection of thymocytes (Lee *et al.,* 2000), and STRL33 is expressed at sufficiently high levels on a subset of CD4-positive T-cells to support virus infection (Sharron *et al.,* 2000). Nonetheless, identifying and studying alternative coreceptors is important because the use of effective CCR5 and CXCR4 antagonists could select for viruses with unusual receptor usage patterns. In this regard, it is worth noting that SIV strains isolated from red-capped mangabeys use CCR2 as their primary receptor, likely due to the fact that most red-capped mangabeys are CCR5-negative due to an inactivating polymorphism, providing a striking example of unexpected coreceptor use in the face of strong selective pressure (Chen *et al.,* 1998). Therefore, it is worth determining which alternative coreceptors are expressed on CD4-positive cell types at levels sufficiently high to support virus infection.

Finally, it is important to ask if CD4 and an appropriate coreceptor are sufficient to support Env-mediated membrane fusion. In favor of this argument is the fact that expression of CD4 and coreceptor in heterologous cell types, including cells from multiple species, invariably makes them targets for Env-mediated membrane fusion. Arguments against this include studies suggesting that glycosphingolipids may play an important role in the HIV-1 membrane fusion reaction, though these studies rely on the use of lipid synthesis inhibitors, making it difficult to control for nonspecific effects (Hug *et al.,* 2000). However, the need for specific lipid types in virus-membrane fusion is not unprecedented. Alphaviruses have an absolute requirement for both cholesterol and sphingomyelin in the target membrane (Kielian, 1995). At present, the role of specific lipids in the HIV-1 fusion process is simply not well understood. Ultimately, reconstitution of the fusion system in artificial membranes will be required to rigorously address the role of specific lipid types in the HIV-1 infection process.

PUTTING THE PIECES TOGETHER

A diagram depicting a model for HIV-1 entry is shown in Fig. 1. CD4 binding triggers conformational changes in Env that enable it to interact with a coreceptor (Lapham *et al.,* 1996; Trkola *et al.,* 1996; Wu *et al.,* 1996). The conformational change appears to result in the formation or exposure of a highly conserved region in gp120 that lies between the bases of the V1/2 and V3 loops and that has been implicated in CCR5 binding (Rizzuto *et al.,* 1998). Given the highly conserved nature of this region, it is likely that this domain also interacts with CXCR4. Coreceptor binding is thought to be the final trigger that results in dramatic structural rearrangements in the gp41 subunit that lead to membrane fusion. The most widely accepted model posits that coreceptor binding leads to the formation of a triple-stranded coiled-coil that enables the hydrophobic fusion peptide at the amino terminus of gp41 to insert into the target cell membrane, making

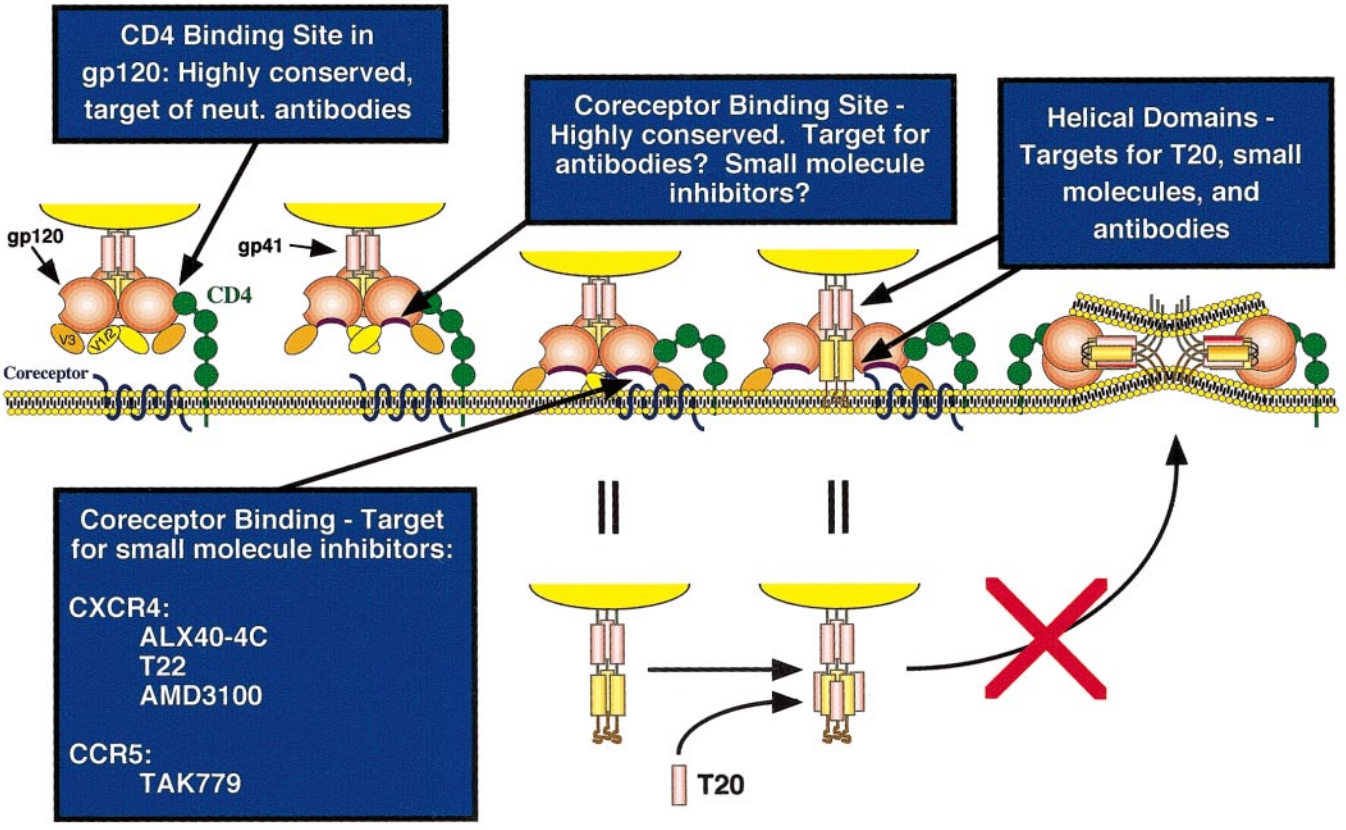

FIG. 1. Targets of opportunity: Inhibition of HIV-1 entry. Binding of CD4 to gp120 leads to exposure of the highly conserved coreceptor binding site in gp120. This domain and the CD4 binding region in gp120 are potential targets for neutralizing antibodies. Binding of gp120 to coreceptor can be inhibited by a variety of small-molecule inhibitors, four of which have been described to date. Coreceptor binding is believed to trigger additional conformational changes in gp41 including the formation of a triple-stranded coiled-coil with insertion of the fusion peptide into the cell membrane. The transition from the triple-stranded coiled-coil to the six helix bundle conformation is the proximal cause of membrane fusion and can be inhibited by T20. T20 is a peptide based on the second helical domain of gp41 that by binding to the first helical domain blocks formation of the six helix bundle.

gp41 an integral component of both the viral and cellular membranes. The triple-stranded coiled-coil then bends back on itself, forming a six helix bundle in which the gp41 fusion peptide and transmembrane domain are at the same end (Chan *et al.,* 1997; Weissenhorn *et al.,* 1997). A recent study by Melikyan *et al.* has shown that it is the transition of gp41 from the coiled-coil to the six helix bundle that is the proximal cause of membrane fusion (Melikyan *et al.,* 2000). This structural transition shares much in common with other viral membrane fusion proteins, including those from orthomyxoviruses, filoviruses, paramyxoviruses, and other retroviruses (Chan and Kim, 1998; Skehel and Wiley, 1998).

TRIGGERING RECONSIDERED

Biological membranes are inherently stable structures that are not prone to fusion, and membrane fusion is tightly controlled with regards to time and place. Clearly, viruses have evolved proteins that efficiently overcome the barriers to membrane fusion. Viral Env proteins appear to exist as metastable structures that, when appropriately triggered, undergo a series of conformational changes that ultimately result in a significant change in free energy (Hernandez *et al.,* 1996). The fusion activity of many enveloped viruses is triggered by acid pH, which is provided by endosomes or lysosomes following internalization of virus through normal endocytic processes (Helenius *et al.,* 1980). There are several advantages to acid pH-dependent virus entry. First, save for those viruses that pass through the gut, the only time a virus will encounter an acidic environment is when it is inside a viable cell, ensuring that fusion does not occur prematurely. Second, endosomes and lysosomes are generally located near the center of the cell. By fusing with the limiting membranes of these organelles, the virus's genetic material enters the cytoplasm in close proximity to the nucleus, the immediate destination for most virus types. Third, acidification is likely to be a synchronous process. All viral fusion proteins, whether they are engaged by receptor or not, are subjected to acidification at the same time, increasing the likelihood that a sufficient

number of viral Env proteins will undergo the conformational changes needed to elicit fusion. The highly cooperative nature of the fusion reaction makes this an efficient way in which to insure kinetic as well as spatial cooperativity: the closely packed viral spike proteins all undergo appropriate conformational changes within a short period of time. Indeed, acidification of membrane-bound influenza virus results in lipid mixing within seconds (Hernandez *et al.,* 1996).

In contrast to acid-activated viruses, viruses that fuse at neutral pH as a consequence of receptor binding are subject to a number of potentially rate-limiting steps that could impact fusion kinetics as well as efficiency, making this a more asynchronous and perhaps less efficient process. In the case of HIV-1, this may provide significant opportunities to develop anti-viral agents that target not the native viral Env protein, but rather the structural intermediates of the fusion process. Indeed, a small peptide inhibitor of the fusion process has already been tested *in vivo* with impressive results, indicating that structural intermediates of the fusion process can be targeted (Kilby *et al.,* 1998). In addition, some of the domains in Env involved in membrane fusion are highly conserved, such as the coreceptor binding site and some regions in gp41, and so could be targets of neutralizing antibodies. Despite the promise of targeting the entry pathway, there are many questions that have yet to be answered. How many receptor binding events, for example, are needed to activate Env trimers and how many trimers are needed to form a fusion pore? How long-lived are the structural intermediates of the fusion process that are drug targets, and can their appearance be prolonged? Does coreceptor binding trigger formation of the triple-stranded coiled-coil, the six helix bundle, or both? Clarifying the steps of HIV-1 entry can only help in the design of immunogens and antiviral agents that target conserved regions of Env that become exposed only during the process of membrane fusion.

MEMBRANE FUSION IS COOPERATIVE

Membrane fusion elicited by viral Env proteins exhibits cooperativity at several levels. Cooperativity has been studied in most detail with the influenza hemagglutinin (HA), where it is estimated that from 3 to 6 HA trimers are needed to form a fusion pore (Ellens *et al.,* 1990; Hernandez *et al.,* 1996). Since the trimers need to be in close proximity to each other, spatial cooperativity is also required. This is clearly not a problem in the context of virus infection in which the viral spike proteins are closely packed, but could limit fusion when cell surface HA proteins mediate fusion with adjoining cells. Indeed, surface density of spike proteins impacts the efficiency of membrane fusion. Intratrimer cooperativity also occurs, since it appears that activation of an HA trimer involves all three subunits simultaneously (Boulay *et al.,* 1988). Finally, as noted above, there is kinetic cooperativity. Upon acid activation, HA trimers become rapidly inactivated if membrane fusion does not occur (Hernandez *et al.,* 1996). Thus, to form a fusion pore, a sufficient number of fusion proteins must be in close proximity, and they must be triggered to undergo conformational changes within a kinetic window that is likely to vary depending on the virus type and perhaps even virus strain.

Only recently has the role of cooperativity in HIV-1 Env mediated membrane fusion been addressed. In a recent study by Kabat and co-workers, the consequences of coreceptor expression levels on virus infection were carefully studied (Kuhmann *et al.,* 2000). They found a nonlinear relationship between CCR5 density and virus infection, leading to an estimate of 6 CCR5 molecules being needed to form a fusion pore. While this estimate depends on several assumptions, the number is certainly compatible with what is known about influenza HA-mediated membrane fusion. We have recently investigated the role of cooperativity in the activation of individual HIV-1 Env trimers. Our results indicate that three CD4 binding events are needed to efficiently activate HIV-1 Env trimers, consistent with an earlier study by Layne *et al.* showing that multimeric CD4 binding is required for HIV-1 infection (Layne *et al.,* 1990). In addition, it appears that multiple coreceptor binding events per trimer are also needed for fusion to occur efficiently.

There are some interesting implications associated with the requirement for multiple HIV-1 Env trimers, and multiple receptor binding events, for the membrane fusion reaction. It logically follows that receptor density will play a role in governing the rate and efficiency of membrane fusion. In addition, since both gp120–CD4 and gp120–coreceptor binding events are fully reversible, we hypothesize that Env trimers must simultaneously engage multiple receptors in order to be activated. Therefore, Env-receptor affinity may also govern the rate and efficiency of membrane fusion. If true, one consequence of using coreceptor antagonists is that receptor density will be reduced, fusion kinetics prolonged, and the structural intermediates of the fusion process longer lived, making the virus potentially more susceptible to compounds like T20 as well as antibodies to conserved regions that are exposed transiently during the fusion process.

IMPLICATIONS OF COOPERATIVITY: RECEPTOR DENSITY

If virus entry requires multiple receptor binding events, it logically follows that receptor density will influence virus infection. Most *in vitro* assays that have been used to assess the types of coreceptors used by virus strains typically express tens of thousands of copies of CD4, CCR5, or CXCR4. Under these conditions, receptor den-

sity is not limiting for virus infection. Primary T-cells and macrophages, however, typically express fewer than 10,000 copies of CCR5 or CXCR4, with expression levels being dependent upon the donor and growth conditions (Lee *et al.,* 1999b). By contrast, CD4 is expressed at much higher levels, with an average of approximately 65,000 molecules per CD4-positive T-cell (Lee *et al.,* 1999b; Lenkei and Andersson, 1995). Therefore, in the context of the primary cell types most commonly infected by HIV-1, coreceptor levels are more likely to influence virus infection than are levels of CD4.

Several studies have investigated the role of coreceptor expression levels in HIV-1 and SIV infection. Platt *et al.* showed that if CD4 is expressed at high levels, very low levels of CCR5 and CXCR4 are needed to support virus infection (Platt *et al.,* 1997). Since CD4 binding triggers exposure of the coreceptor binding site, high levels of CD4 might enable Env to interact with CCR5 or CXCR4 more efficiently. Other studies have confirmed this finding for both HIV-1 and SIV (Edinger *et al.,* 1999; Sharron *et al.,* 2000).

While CCR5 and CXCR4 can support virus infection even at very low levels of expression, this is not always true for alternative coreceptors. In the case of STRL33, for example, there appears to be a threshold level of receptor expression below which infection does not occur, with the threshold varying for different virus strains (Sharron *et al.,* 2000). Some viruses can use this receptor only at expression levels that far exceed those found *in vivo.* There is also evidence that CCR3 functions as an HIV-1 coreceptor only at high levels of receptor expression (Rucker *et al.,* 1997). By contrast, the APJ coreceptor behaves like CCR5 and CXCR4 in that it exhibits coreceptor activity even at low levels of expression (Puffer *et al.,* 2000). It will be important to determine if other viral coreceptors function at the low levels of expression typically found *in vivo,* or whether they function only when overexpressed.

IMPLICATIONS OF COOPERATIVITY: RECEPTOR AFFINITY

If multiple receptor binding events are needed to activate individual HIV-1 Env trimers, it logically follows that Envs that exhibit higher affinities for their receptors will be able to elicit membrane fusion more quickly than Env proteins that exhibit poor receptor binding. What then is known about the affinity of different HIV-1 Env proteins for CD4 and the coreceptors? In general, it appears that Env-CD4 binding constants are less variable than Env-coreceptor binding constants. Env-CD4 affinities are often below 10 nM, though there is some variation (Moore, 1990; Moore *et al.,* 1990, 1992). The high resolution structure of a gp120 core fragment complexed with CD4 shows that many of the contact residues in gp120 are highly conserved between divergent virus strains, providing an explanation for this modest variability (Kwong *et al.,* 1998).

There is considerable variation between Envs with regards to coreceptor binding affinities, though it should be noted that studies in which direct measurements of gp120 binding to CCR5 and CXCR4 have been made are relatively few in number. Published binding constants for gp120–CCR5 interactions range from approximately 4 to 15 nM (Doranz *et al.,* 1999). In general, it appears that addition of soluble CD4 to R5 gp120 proteins results in readily detectable binding of the CD4–gp120 complex to the surface of CCR5-positive cells. By contrast, direct binding of X4 gp120s to CXCR4 has been very difficult to detect using equilibrium binding assays (Baik *et al.,* 1999; Doranz *et al.,* 1999). By incorporating CXCR4 into retrovirus particles and attaching these to the surface of an optical biosensor, we were able to study gp120–CXCR4 binding in real time. For the commonly studied gp120 from the HxB strain, a binding constant of approximately 500 nM was measured, explaining the difficulty in detecting gp120–CXCR4 binding using equilibrium binding assays (Hoffman *et al.,* 2000). With these assays, the rapid off-rate exhibited by gp120 results in low or undetectable signals. In addition, R5X4 proteins appear to interact weakly with both CCR5 and CXCR4, suggesting that a consequence of broadened tropism is reduced affinity for both of the major coreceptors (Baik *et al.,* 1999).

Taken together, the binding studies published to date indicate that Env-coreceptor binding constants can vary by two orders of magnitude. Nonetheless, weak coreceptor interactions are compatible with virus infection *in vitro* and *in vivo,* as evidenced by pathogenic X4 and R5X4 SHIV and by the recovery of primary R5X4 and X4 virus strains that bind weakly to coreceptors. Are there any practical consequences associated with reduced coreceptor affinity? It is important to note that while Env-coreceptor interactions are typically studied with monomeric gp120, the viral Env is a trimer, each virus has multiple trimers, and virus is tethered to the cell surface by high affinity interactions with CD4. Therefore, the off-rate of gp120 from CXCR4 may have negligible consequences in terms of virus dissociation. Where coreceptor affinity may be important is when cells have relatively low levels of coreceptor on the cell surface. If two or three simultaneous coreceptor binding events are needed to activate a trimer, then a fast off-rate would make it less likely that at any give time an Env trimer will be fully engaged by receptors, reducing the rate of the conformational change and hence membrane fusion.

VISIBLE AND INVISIBLE RECEPTORS: RECEPTOR CONFORMATION

The seven transmembrane domain chemokine receptors transduce information across the plasma membrane

upon ligand binding that results in the generation of specific signaling events. Given this, it is not surprising the 7TM receptors in general can exist in distinct conformational states. The chemokine receptors are no exception to this, and antigenically distinct conformations of CCR5 and CXCR4 have been described. In the case of CXCR4, a panel of monoclonal antibodies was used to stain various B- and T-cell lines under saturating conditions to measure CXCR4 expression. Though the absolute levels of CXCR4 varied between B-cell lines, there was no antigenic heterogeneity: each antibody recognized approximately the same level of CXCR4 on a given cell type. However, on T-cell lines a different pattern emerged, in which two reactivity patterns were obtained. One group of antibodies consistently recognized CXCR4 more efficiently than other antibodies regardless of which T-cell line was examined. These differences in antibody reactivity were not due to differences in antibody affinity for CXCR4, but rather reflected conformational heterogeneity of CXCR4 in T-cell lines. The same pattern of reactivity was seen on primary T-cells. Conformational heterogeneity has also been observed for CCR5, though this does not exhibit the same cell type dependence as was seen for CXCR4 (Lee *et al.,* 1999a).

The mechanisms responsible for CCR5 and CXCR4 antigenic heterogeneity are not known, nor are the consequences for coreceptor or chemokine receptor function. Experiments performed to date suggest that posttranslational modifications such as N-linked glycosylation and sulfation are not responsible for these differences. It will be interesting to determine if all forms of CCR5 and CXCR4 can function as chemokine and coreceptors, and if these differences can help explain why some viruses can use CXCR4 to infect macrophages whereas others cannot. One practical consequence of these findings is that use of a single antibody to study receptor expression could lead to underestimation of receptor number. The monoclonal antibody 12G5 is by far the most commonly used reagent to study CXCR4 expression, yet this antibody recognizes only a subset of available CXCR4 conformations on T-cells. Alteration of receptor conformation represents a potential mechanism by which small molecules may inhibit coreceptor function. The first small molecule inhibitor of CCR5, TAK779 (Baba *et al.,* 1999), efficiently blocks HIV-1 infection via this receptor and blocks gp120–CCR5 binding. However, a recent study by Dragic and co-workers showed that TAK779 binds to a hydrophobic pocket formed largely by the transmembrane domain regions of CCR5 (Dragic *et al.,* 2000). Since gp120 binding to CCR5 is mediated by the amino terminal domain of CCR5 and also by interactions with the extracellular loops (Baik *et al.,* 1999; Dragic *et al.,* 1998), how then does TAK779 block gp120 binding? It is intriguing to speculate that TAK779, by binding to this hydrophobic pocket, alters CCR5 conformation such that it no longer binds to gp120 or does so poorly. Antibodies that recognize specific receptor conformations could potentially be used to determine if TAK779 does in fact alter CCR5 conformation. In summary, the HIV-1 coreceptors exist in multiple conformations that could influence viral infectivity and the use of small molecule inhibitors.

OPPORTUNITIES TO INTERVENE

The viral entry pathway affords many potential opportunities to block virus infection (Fig. 1). Small molecule inhibitors of both CCR5 and CXCR4 have been described, with more under development (Baba *et al.,* 1999; Donzella *et al.,* 1998; Doranz *et al.,* 1997; Murakami *et al.,* 1997; Schols *et al.,* 1997). Given the success of the pharmaceutical industry in targeting other 7TM receptors and the protection against virus infection afforded by the Δ32-ccr5 polymorphism, this is a particularly exciting area of research. Env can also be targeted. The small peptide inhibitor T20 prevents conversion of gp41 into the six-helix bundle conformation and has been shown to dramatically reduce viral load *in vivo* (Kilby *et al.,* 1998). The structure of the gp41 core fragment also reveals the presence of a hydrophobic pocket that is a potential target for small molecule fusion inhibitors (Eckert *et al.,* 1999). Potentially, both the CD4 and coreceptor binding sites in gp120 could be drug targets as well. Another approach is to develop immunogens that elicit antibodies to the conserved regions of Env that are involved in the entry process. The coreceptor binding site in gp120, the CD4 binding site, and regions in gp41 are all attractive targets. There is now considerable interest in generating modified forms of Env that will present these domains more efficiently. Desrosiers and co-workers found that eliminating two N-linked glycosylation sites in the V1/V2 region of SIVmac239 Env resulted in a virus that was both neutralization sensitive and that also elicited antibodies capable of neutralizing the fully glycosylated parental virus which is otherwise extraordinarily difficult to neutralize (Reitter *et al.,* 1998). Genetically triggered forms of Env have been described that are CD4-independent, being able to interact directly with either CCR5 or CXCR4 to infect cells (Hoffman *et al.,* 1999; Kolchinsky *et al.,* 1999; LaBranche *et al.,* 1999). At least some of these Envs are neutralization sensitive, suggesting that conserved neutralization determinants are exposed (Hoffman *et al.,* 1999). Whether genetically triggered forms of Env or other modified immunogens will elicit antibodies capable of neutralizing primary isolates is not yet known, but is a promising area of research.

SUMMARY

The identification of the receptors needed by the HIV-1 Env protein to mediate membrane fusion illustrates the importance of characterizing the entry pathways of viruses in general. The discovery of the HIV-1 coreceptors

has greatly influenced our understanding of viral tropism and pathogenesis and leads to the identification of genetic factors that influence virus transmission and disease progression. The challenge for the future will not be so much to determine which viruses use which receptors, but rather how these receptors are used and how this correlates with viral tropism and pathogenicity. Increased understanding of the entry pathway will also accelerate development of entry inhibitors, which represent an entirely new class of antiviral agents. Potentially, these approaches could be taken for other important viral pathogens as well.

REFERENCES

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., and Berger, E. A. (1996). CC CKR5: A RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272,** 1955–1958.

Baba, M., Nishimura, O., Kanzaki, N., Okamoto, M., Sawada, H., Iizawa, Y., Shiraishi, M., Aramaki, Y., Okonogi, K., Ogawa, Y., Meguro, K., and Fujino, M. (1999). A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV− activity. *Proc. Natl. Acad. Sci. USA* **96,** 5698–5703.

Baik, S., Doms, R. W., and Doranz, B. J. (1999). HIV and SIV gp120 binding does not predict coreceptor function. *Virology* **259,** 267–273.

Bazan, H. A., Alkhatib, G., Broder, C. C., and Berger, E. A. (1998). Patterns of CCR5, CXCR4, and CCR3 usage by envelope glycoproteins from human immunodeficiency virus type 1 primary isolates. *J. Virol.* **72,** 4485–4491.

Berger, E. A., Doms, R. W., Fenyö, E. M., Korber, B. T. M., Littman, D. R., Moore, J. P., Sattentau, Q. J., Schuitemaker, H., Sodroski, J., and Weiss, R. A. (1998). A new classification for HIV-1. *Nature* **391,** 240.

Boulay, F., Doms, R. W., Webster, R. G., and Helenius, A. (1988). Post-translational oligomerization and cooperative acid activation of mixed influenza hemagglutinin trimers. *J. Cell Biol.* **106,** 629–639.

Cayabyab, M., Karlsson, G., Etemad-Moghadam, B., Hofmann, W., Steenbeke, T., Halloran, M., Fanton, J., Axthelm, M., Letvin, N., and Sodroski, J. (1999). Changes in human immunodeficiency virus type 1 envelope glycoproteins responsible for the pathogenicity of a multiply passaged simian–human immunodeficiency virus (SHIV-HXBc2). *J. Virol.* **73,** 976–984.

Chan, D. C., Fass, D., Berger, J. M., and Kim, P. S. (1997). Core structure of gp41 from the HIV envelope glycoprotein. *Cell* **89,** 263–273.

Chan, D. C., and Kim, P. S. (1998). HIV entry and its inhibition. *Cell* **93,** 681–684.

Chen, Z., Kwon, D., Jin, Z., Monard, S., Telfer, P., Jones, M., Lu, C., Aguilar, R., Ho, D., and Marx, P. (1998). Natural infection of a homozygous delta-24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. *J. Exp. Med.* **188,** 2057–2065.

Choe, H., Farzan, M., Konkel, M., Martin, K., Sun, Y., Marcon, L., Cayabyab, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J. (1998). The orphan seven-transmembrane receptor Apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J. Virol.* **72,** 6113–6118.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C., and Sodroski, J. (1996). The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85,** 1135–1148.

Connor, R. I., Sheridan, K. E., Ceradini, D., Choe, S., and Landau, N. R. (1997). Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. *J. Exp. Med.* **185,** 621–628.

Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Goedert, J. J., Buchbinder, S. P., Vittinghoff, E., Gomperts, E., Donfield, S., Vlahov, D., Kaslow, R., Saah, A., Rinaldo, C., Detels, R., and O'Brien, S. J. (1996). Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science* **273,** 1856–1862.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Marzio, P. D., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381,** 661–666.

Deng, H., Unutmaz, D., Kewalramani, V. N., and Littman, D. R. (1997). Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* **388,** 296–300.

Dittmar, M. T., McKnight, K., Simmons, G., Clapham, P. R., Weiss, R. A., and Simmonds, P. (1997). HIV-1 tropism and coreceptor use. *Nature* **385,** 495–496.

Doms, R. W., Edinger, A., and Moore, J. (1999). Coreceptor use by primate lentiviruses. *In* "Human Retroviruses and AIDS" (B. Korber, B. Foley, T. Leitner, G. Myers, B. Hahn, F. McCutchan, J. Mellors, and C. Kuiken, Eds.). Los Alamos National Laboratory: Theoretical Biology and Biophysics, Los Alamos, NM.

Donzella, G. A., Schols, D., Lin, S. W., Esté, J. A., Nagashima, K. A., Maddon, P. J., Allaway, G. P., Sakmar, T. P., Henson, G., De Clerq, E., and Moore, J. P. (1998). AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat. Med.* **4**(1), 72–77.

Doranz, B. J., Baik, S. S. W., and Doms, R. W. (1999). Use of a gp120 binding assay to dissect the requirements and kinetics of human immunodeficiency virus fusion events. *J. Virol.* **73,** 10346–10358.

Doranz, B. J., Grovit-Ferbasi, K., Sharron, M. P., Mao, S. H., Goetz, M. B., Daar, E. S., Doms, R. W., and O'Brien, W. A. (1997). A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. *J. Exp. Med.* **186,** 1395–1400.

Doranz, B. J., Orsini, M. J., Turner, J. D., Hoffman, T. D., Berson, J. F., Lu, Z.-H., Hoxie, J. A., Peiper, S. C., Brass, L. F., and Doms, R. W. (1999). Identification of CXCR4 domains that support coreceptor and chemokine receptor functions. *J. Virol.* **73,** 2752–2761.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Doms, R. W. (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85,** 1149–1158.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P., and Paxton, W. A. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381,** 667–673.

Dragic, T., Trkola, A., Lin, S. W., Nagashima, K. A., Kajumo, F., Zhao, L., Olson, W. C., Wu, L., Mackay, C. R., Allaway, G. P., Sakmar, T. P., Moore, J. P., and Maddon, P. J. (1998). Amino-terminal substitutions in the CCR5 co-receptor impair gp120 binding and human immunodeficiency virus type 1 entry. *J. Virol.* **72,** 279–285.

Dragic, T., Trkola, A., Thompson, D. A. D., Cormier, E. G., Kajumo, F. A., Maxwell, E., Lin, S. W., Ying, W., Smith, S. O., Sakmar, T. P., and Moore, J. P. (2000). A binding pocket for a small molecule inhibitor of HIV entry within the transmembrane helices of CCR5. *Proc. Natl. Acad. Sci. USA* **97,** 5639–5644.

Eckert, D. M., Malashkevich, V. N., Hong, L. H., Carr, P. A., and Kim, P. S. (1999). Inhibiting HIV-1 entry: Discovery of D-peptide inhibitors that target the gp41 coiled-coil pocket. *Cell* **99,** 103–115.

Edinger, A. L., Blanpain, C., Kunstman, K. J., Wolinsky, S. M., Parmentier, M., and Doms, R. W. (1999). Functional dissection of CCR5 coreceptor function through the use of CD4-independent simian immunodeficiency virus strains. *J. Virol.* **73,** 4062–4073.

Edinger, A. L., Hoffman, T. L., Yi, Y., Sharron, M., Collman, R. G., Mitrovic, B., Faulds, D., Hesselgesser, J., Horuk, R., and Doms, R. W. (1998). An orphan seven transmembrane domain receptor expressed widely in brain functions as a coreceptor for HIV-1 and SIV. *J. Virol.* **72,** 7934–7940.

Ellens, H., Bentz, J., Mason, D., Zhang, F., and White, J. M. (1990). Fusion of influenza hemagglutinin-expressing fibroblasts with glycophorin-

bearing liposomes: Role of hemagglutinin surface density. *Biochemistry* **29,** 9697–9707.

Etemad-Moghadam, B., Sun, Y., Nicholson, E. K., Fernandes, M., Liou, K., Gomila, R., Lee, J., and Sodroski, J. (2000). Envelope glycoprotein determinants of increased fusogenicity in a pathogenic simian–human immunodeficiency virus (SHIV-KB9) passaged *in vivo*. *J. Virol.* **74,** 4433–4440.

Farzan, M., Choe, H., Martin, K., Marcon, L., Hofmann, W., Karlsson, G., Sun, Y., Barrett, P., Marchand, N., Sullivan, N., Gerard, N., Gerard, C., and Sodroski, J. (1997). Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *J. Exp. Med.* **186,** 405–411.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996). HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane domain, G-protein coupled receptor. *Science* **272,** 872–877.

Geijtenbeek, T. B. H., Kwon, D. S., Torensma, R., Vliet, S. J. v., Duijnhoven, G. C. F. v., Middel, J., Cornelissen, I. L. M. H. A., Nottet, H. S. L. M., Kewalramani, V. N., Littman, D. R., Figdor, C. G., and Kooyk, Y. v. (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100,** 587–597.

Helenius, A., Kartenbeck, J., Simons, K., and Fries, E. (1980). On the entry of Semliki Forest virus into BHK-21 cells. *J. Cell Biol.* **84,** 404–420.

Hernandez, L. D., Hoffman, L. R., Wolfsberg, T. G., and White, J. M. (1996). Virus–cell and cell–cell fusion. *Annu. Rev. Dev. Cell Biol.* **12,** 627–661.

Hoffman, T. L., Canziani, G., Rucker, J., and Doms, R. W. (2000). Use of a novel biosensor assay to measure ligand interactions with membrane proteins. *Proc. Natl. Acad. Sci. USA,* in press.

Hoffman, T. L., LaBranche, C., Zhang, W., Canziani, G., Robinson, J., Chaiken, I., Hoxie, J. A., and Doms, R. W. (1999). Stable exposure of the coreceptor binding site in a CD4-independent HIV-1 envelope protein. *Proc. Natl. Acad. Sci. USA* **96,** 6359–6364.

Hug, P., Lin, H.-M. J., Korte, T., Xao, X., Dimitrov, D. S., Wang, J. M., Puri, A., and Blumenthal, R. (2000). Glycosphingolipids promote entry of a broad range of human immunodeficiency virus type 1 isolates into cell lines expressing CD4, CXCR4, and/or CCR5. *J. Virol.* **74,** 6377–6385.

Karlsson, G. B., Halloran, M., Schenten, D., Lee, J., Racz, P., Tenner-Racz, K., Manola, J., Gelman, R., Etemad-Moghadam, B., Desjardins, E., Wyatt, R., Gerard, N. P., Marcon, L., Margolin, D., Fanton, J., Axthelm, M. K., Letvin, N. L., and Sodroski, J. (1998). The envelope glycoprotein ectodomains determine the efficiency of CD4+ T lymphocyte depletion in simian–human immunodeficiency virus-infected macaques. *J. Exp. Med.* **188,** 1159–1171.

Kielian, M. (1995). Membrane fusion and the alphavirus life cycle. *Adv. Virus Res.* **45,** 113–151.

Kilby, J. M., Hopkins, S., Venetta, T. M., DiMassimo, B., Cloud, G. A., Lee, J. Y., Alldredge, L., Hunter, E., Lambert, D., Bolognesi, D., Matthews, T., Johnson, M. R., Nowak, M. A., Shaw, G. M., and Saag, M. S. (1998). Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat. Med.* **4,** 1302–1307.

Kolchinsky, P., Mirzabekov, T., Farzan, M., Kiprilov, E., Cayabyab, M., Mooney, L., Choe, H., and Sodroski, J. (1999). Adaptation of a CCR5 using primary HIV-1 isolate for CD4-independent replication. *J. Virol.* **73,** 8120–8126.

Kuhmann, S. E., Platt, E. J., Kozak, S. L., and Kabat, D. (2000). Cooperation of multiple CCR5 coreceptors is required for infections by human immunodeficiency virus type I. *J. Virol.* **74,** 7005–7015.

Kwong, P. D., Wyatt, R., Robinson, J., Sweet, R. W., Sodroski, J., and Hendrickson, W. A. (1998). Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393,** 648–659.

LaBranche, C., Hoffman, T. L., Romano, J., Haggarty, B. S., Matthews, T. J., Doms, R. W., and Hoxie, J. A. (1999). Determinants of CD4-independence for HIV-1/IIIBx map outside regions required for coreceptor specificity. *J. Virol.* **73,** 10310–10319.

Lapham, C. K., Ouyang, J., Chandrasekhar, B., Nguyen, N. Y., Dimitrov, D. S., and Golding, H. (1996). Evidence for cell-surface association between Fusin and the CD4–gp120 complex in human cell lines. *Science* **274,** 602–605.

Layne, S. P., Merges, M. J., Dembo, M. B., Spouge, J. L., and Nara, P. L. (1990). HIV requires multiple gp120 molecules for CD4-mediated infection. *Nature* **346,** 277–279.

Lee, B., Sharron, M., Blanpain, C., Doranz, B. J., Vakili, J., Setoh, P., Berg, E., Liu, G., Guy, H. R., Durell, S. R., Parmentier, M., Chang, C. N., Price, K., Tsang, M., and Doms, R. W. (1999a). Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. *J. Biol. Chem.* **274,** 9617–9626.

Lee, B., Sharron, M., Montaner, L., Weissman, D., and Doms, R. (1999b). Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. *Proc. Nat. Acad. Sci. USA* **96,** 5215–5220.

Lee, S., Tiffany, H. L., King, L., Murphy, P. M., Golding, H., and Zaitseva, M. B. (2000). CCR8 on human thymocytes functions as a human immunodeficiency virus type 1 coreceptor. *J. Virol.* **74,** 6946–6952.

Lenkei, R., and Andersson, B. (1995). Determination of the antibody binding capacity of lymphocyte membrane antigens by flow cytometry in 58 blood donors. *J. Immunol. Methods* **183,** 267–277.

Liao, F., Alkhatib, G., Peden, K. W. C., Sharma, G., Berger, E. A., and Farber, J. M. (1997). STRL33, a novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* **185,** 2015–2023.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A., and Landau, N. R. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86,** 367–377.

Liu, Z., Muhkerjee, S., Sahni, M., McKormick-Davis, C., Leung, K., Li, Z., Gattone, V., Tian, C., Doms, R. W., Hoffman, T. L., Raghavan, R., Narayan, O., and Stephens, E. (1999). Derivation and biological characterization of a molecular clone of SHIV KU-2 that causes AIDS, neurological disease, and renal disease in rhesus macaques. *Virology* **260,** 295–307.

McKnight, A., Wilkinson, D., Simmons, G., Talbot, S., Picard, L., Ahuja, M., Marsh, M., Hoxie, J. A., and Clapham, P. R. (1997). Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. *J. Virol.* **71,** 1692–1696.

Melikyan, G. B., Markosyan, R. M., Hemmati, H., Delmedico, M. K., Lambert, D. M., and Cohen, F. S. (2000). The transition of HIV-1 gp41 into a six-helix bundle configuration induces membrane fusion. *J. Cell Biol.,* in press.

Mondor, I., Ugolini, S., and Sattentau, Q. J. (1998). Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. *J. Virol.* **72,** 3623–3634.

Moore, J. P. (1990). Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbent assay: HIV-2 has a 25-fold lower affinity than HIV-1 for soluble CD4. *AIDS* **4,** 297–305.

Moore, J. P., McKeating, J. A., Huang, Y., Ashkenazi, A., and Ho, D. D. (1992). Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. *J. Virol.* **66,** 235–243.

Moore, J. P., McKeating, J. A., Jones, I. M., Stephens, P. E., Clements, G., Thomson, S., and Weiss, R. A. (1990). Characterization of recombinant gp120 and gp160 from HIV-1: binding to monoclonal antibodies and soluble CD4. *AIDS* **4,** 307–315.

Moriuchi, H., Moriuchi, M., Arthos, J., Hoxie, J., and Fauci, A. S. (1997). Promonocytic U937 subclones expressing CD4 and CXCR4 are re-

sistant to infection with and cell-to-cell fusion by T-cell-tropic human immunodeficiency virus type 1. *J. Virol.* **71,** 9664–9671.

Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fujii, N., Tamamura, H., Yoshida, N., Waki, M., Matsumoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Nagasawa, T. (1997). A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. *J. Exp. Med.* **186**(8), 1389–1393.

Platt, E. J., Wehrly, K., Kuhmann, S. E., Chesebro, B., and Kabat, D. (1997). Effects of CCR5 and CD4 cell surface concentrations by macrophage-tropic isolates of human immunodeficiency virus type 1. *J. Virol.* **72,** 2855–2864.

Puffer, B. A., Sharron, M., Coughlan, C. M., McManus, C., Lee, B., Horuk, R., Dietz, M., Tsang, M., and Doms, R. W. (2000). Expression and coreceptor function of APJ for primate immunodeficiency viruses. *Virology,* in press.

Rana, S., Besson, G., Cook, D. G., Rucker, J., Smyth, R. J., Yi, Y., Turner, J. D., Guo, H.-H., Du, J.-G., Peiper, S. C., Lavi, E., Samson, M., Libert, F., Liesnard, C., Vassart, G., Doms, R. W., Parmentier, M., and Collman, R. G. (1997). Role of CCR5 in infection of primary macrophages and lymphocytes by macrophage-tropic strains of human immunodeficiency virus: Resistance to patient-derived and prototype isolates resulting from the Δ*ccr5* mutation. *J. Virol.* **71,** 3219–3227.

Reeves, J. D., McKnight, A., Potempa, S., Simmons, G., Gray, P. W., Power, C. A., Wells, T., Weiss, R. A., and Talbot, S. J. (1997). CD4-independent infection by HIV-2 (ROD/B): Use of the 7-transmembrane receptors CXCR-4, CCR-3, and V28 for entry. *Virology* **231,** 130–134.

Reimann, K. A., Li, J. T., Voss, G., Lekutis, C., Tenner-Racz, K., Racz, P., Lin, W., Montefiori, D. C., Lee-Parritz, D. E., Lu, Y., Collman, R. G., Sodroski, J., and Letvin, N. L. (1996). An env gene derived from a primary human immunodeficiency virus type 1 isolate confers high *in vivo* replicative capacity to a chimeric simian/human immunodeficiency virus in rhesus monkeys. *J. Virol.* **70,** 3198–3206.

Reitter, J. N., Means, R. E., and Desrosiers, R. C. (1998). A role for carbohydrates in immune evasion in AIDS. *Nat. Med.* **4,** 679–684.

Rizzuto, C. D., Wyatt, R., Hernandez-Ramos, N., Sun, Y., Kwong, P. D., Hendrickson, W. A., and Sodroski, J. (1998). A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* **280,** 1949–1953.

Rucker, J., Edinger, A. L., Sharron, M., Samson, M., Lee, B., Berson, J. F., Yi, Y., Collman, R. G., Doranz, B. J., Parmentier, M., and Doms, R. W. (1997). Utilization of chemokine receptors, orphan receptors, and herpesvirus encoded receptors by diverse human and simian immunodeficiency viruses. *J. Virol.* **71,** 8999–9007.

Samson, M., Edinger, A. L., Stordeur, P., Rucker, J., Verhasselt, V., Sharron, M., Govaerts, C., Mollereau, C., Vassart, G., Doms, R. W., and Parmentier, M. (1998). ChemR23, a putative chemoattractant receptor, is expressed in dendritic cells and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* **28,** 1689–1700.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C.-M., Saragosti, S., Lapoumèroulie, C., Cogniaux, J., Forceille, C., Muyldermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collman, R. G., Doms, R. W., Vassart, G., and Parmentier, M. (1996). Resistance to HIV-1 infection of Caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene. *Nature* **382,** 722–725.

Scarlatti, G., Tresoldi, E., Bjorndal, A., Fredriksson, R., Colognesi, C., Deng, H. K., Malnatti, M. S., Plebani, A., Siccardi, A. G., Littman, D. R., Fenyo, E. M., and Lusso, P. (1997). *In vivo* evolution of HIV-1 coreceptor usage and sensitivity to chemokine-mediated suppression. *Nat. Med.* **11,** 1259–1265.

Schmidtmayerova, H., Alfano, M., Nuovo, G., and Burkinsky, M. (1998). Human immunodeficiency virus type 1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-mediated pathway: Replication is restricted at a postentry level. *J. Virol.* **72,** 4633–4642.

Schols, D., Struyf, S., Damme, J. V., Esté, J. A., Henson, G., and Clercq, E. D. (1997). Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J. Exp. Med.* **186,** 1383–1388.

Sharron, M. P., Pöhlmann, S., Price, K., Tsang, M., Kirchoff, F., Doms, R. W., and Lee, B. (2000). Expression and coreceptor activity of STRL33 on primary peripheral blood lymphocytes. *Blood* **96,** 41–49.

Shieh, J. T., Martin, J., Baltuch, G., Malim, M. H., and Gonzalez-Scarano, F. (2000). Determinants of syncytium formation in microglia by human immunodeficiency virus type 1: Role of the V1/V2 domains. *J. Virol.* **74,** 693–701.

Simmons, G., Reeves, J. D., McKnight, A., Dejucq, N., Hibbitts, S., Power, C., Aarons, E., Schols, D., DeKlecq, E., Proudfoot, A. E., and Clapham, P. R. (1998). CXCR4 as a functional coreceptor for human immunodeficiency virus type 1 infection of primary macrophages. *J. Virol.* **72,** 8453–8457.

Skehel, J. J., and Wiley, D. C. (1998). Coiled coils in both intracellular and viral membrane fusion. *Cell* **95,** 871–874.

Trkola, A., Drajic, T., Arthos, J., Binley, J. M., Olson, W. C., Allaway, G. P., Cheng-Mayer, C., Robinson, J., Maddon, P. J., and Moore, J. P. (1996). CD4-dependent, antibody-sensitive interactions between HIV-1 and its coreceptor CCR-5. *Nature* **384,** 184–187.

Verani, A., Pesenti, E., Polo, S., Tresoldi, E., Scarlatti, G., Lusso, P., Siccardi, A. G., and Vercelli, D. (1998). CXCR-4 is a functional coreceptor for infection of human macrophages by CXCR4-dependent primary HIV-1 isolates. *J. Immunol.* **161,** 2084–88.

Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J., and Wiley, D. C. (1997). Atomic structure of the ectodomain from HIV-1 gp41. *Nature* **387,** 426–430.

Wu, L., Gerard, N. P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., Borsetti, A., Cardoso, A. A., Desjardin, E., Newman, W., Gerard, C., and Sodroski, J. (1996). CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* **384,** 179–183.

Wyatt, R., and Sodroski, J. (1998). The HIV-1 envelope glycoproteins: Fusogens, antigens, and immunogens. *Science* **280,** 1884–1888.

Yi, Y., Rana, S., Turner, J. D., Gaddis, N., and Collman, R. G. (1998). CXCR4 is expressed by primary macrophages and supports CCR5-independent infection by dual-tropic but not T-tropic isolates of HIV-1. *J. Virol.* **72,** 772–777.

Zhang, Y.-J., Dragic, T., Cao, Y., Kostrikis, L., Kwon, D. S., Littman, D. R., Kewalramani, V. N., and Moore, J. P. (1998). Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare *in vitro*. *J. Virol.* **72,** 9337–9344.